Reproductive Toxicology 27 (2009) 258–265

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPARα) in the mouse☆

Barbara D. Abbott[a,*], Cynthia J. Wolf[a], Kaberi P. Das[a], Robert D. Zehr[a], Judith E. Schmid[a], Andrew B. Lindstrom[b], Mark J. Strynar[b], Christopher Lau[a]

[a] *Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, United States*
[b] *Human Exposure and Atmospheric Sciences Division, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, United States*

ARTICLE INFO

Article history:
Received 21 March 2008
Received in revised form 12 May 2008
Accepted 16 May 2008
Available online 24 May 2008

Keywords:
Perfluorooctane sulfonate (PFOS)
Developmental toxicity
Peroxisome proliferator activated receptor-alpha (PPAR-α)

ABSTRACT

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are members of a family of perfluorinated compounds. Both are environmentally persistent and found in the serum of wildlife and humans. PFOS and PFOA are developmentally toxic in laboratory rodents. Exposure to these chemicals *in utero* delays development and reduces postnatal survival and growth. Exposure to PFOS on the last 4 days of gestation in the rat is sufficient to reduce neonatal survival. PFOS and PFOA are weak agonists of peroxisome proliferator activated receptor-alpha (PPARα). The reduced postnatal survival of neonatal mice exposed to PFOA was recently shown to depend on expression of PPARα. This study used PPARα knockout (KO) and 129S1/SvlmJ wild type (WT) mice to determine if PPARα expression is required for the developmental toxicity of PFOS. After mating overnight, the next day was designated gestation day (GD) 0. WT females were weighed and dosed orally from GD15 to 18 with 0.5% Tween-20, 4.5, 6.5, 8.5, or 10.5 mg PFOS/kg/day. KO females were dosed with 0.5% Tween-20, 8.5 or 10.5 mg PFOS/kg/day. Dams and pups were observed daily and pups were weighed on postnatal day (PND) 1 and PND15. Eye opening was recorded from PND12 to 15. Dams and pups were killed on PND15, body and liver weights recorded, and serum collected. PFOS did not affect maternal weight gain or body or liver weights of the dams on PND15. Neonatal survival (PND1–15) was significantly reduced by PFOS in both WT and KO litters at all doses. WT and KO pup birth weight and weight gain from PND1 to 15 were not significantly affected by PFOS exposure. Relative liver weight of WT and KO pups was significantly increased by the 10.5 mg/kg dose. Eye opening of PFOS-exposed pups was slightly delayed in WT and KO on PND13 or 14, respectively. Because results in WT and KO were comparable, it is concluded that PFOS-induced neonatal lethality and delayed eye opening are not dependent on activation of PPARα.

Published by Elsevier Inc.

1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are members of a family of perfluorinated compounds and both are environmentally persistent and found in the serum of wildlife and humans [1–6]. The 8-carbon chain compounds, PFOS and PFOA, have chemical properties that make them excellent surfactants. These attributes lead to many industrial and consumer applications, including uses for coatings for fabrics, carpets, paper products, in electroplating, etching, photography, hydraulic fluids, fire-fighting foams, paints, waxes, and adhesives [7–9]. Monitoring studies of environmental media, wildlife, and human tissues found PFOA and PFOS in samples collected from around the world [10–13]. The global production and use of PFOS has declined since the phase

☆ *Disclaimer*: The information in this document has been funded wholly (or in part) by the US Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

* Corresponding author at: NHEERL Building, US Environmental Protection Agency, 2525 East Highway 54, Durham, NC 27713, United States.
Tel.: +1 919 541 2753; fax: +1 919 541 4017.
E-mail address: Abbott.barbara@epa.gov (B.D. Abbott).

0890-6238/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.reprotox.2008.05.061

out of manufacturing of the chemical by its major producer in 2002 [14] and US monitoring studies suggest that the levels of PFOS and PFOA may currently be declining in human blood samples [15,16]. However, these compounds continue to be detected in all matrices sampled and PFOA and PFOS generally are the most likely to be detected and are typically at higher concentrations than other perfluorinated compounds. PFOS and PFOA have been detected in human blood, plasma, liver, seminal fluid, breast milk, and umbilical cord blood [13,17–22]. See Lau et al. [23] for a recent review of the monitoring and toxicity of these compounds.

PFOS and PFOA have been extensively studied for their effects in animals and toxic responses to exposure include loss of body weight, liver toxicity, tumorigenicity, mortality, and developmental toxicity (reviewed by [14,24]). The effects of *in utero* exposure of mice and rats to PFOS include fetal weight reduction, cleft palate, reduced postnatal survival, delayed eye opening, thyroid hormone imbalance (depression of serum T4 and T3), and persistent postnatal growth deficits [25–28]. PFOA also produces reproductive and developmental toxicity in rats and mice [29–31], although the outcomes in rats are affected by a sex-dependent difference in the elimination of PFOA, i.e. female rats have low levels of PFOA in serum compared to male rats [31]. Mice do not have this gender difference and levels of PFOA in serum are similar in the male and female mice. The developmental toxicity of PFOA in mice is similar to that seen after exposure to PFOS, with fetal weight reduction, dose-related full litter resorptions, reduced postnatal survival, delayed eye opening, postnatal growth deficits, sex-specific alterations in pubertal maturation, and persistent post-weaning growth deficits [31]. Cross-foster studies for both PFOS and PFOA demonstrated that the postnatal effects were not a consequence of altered maternal behaviors [25,26,32,33]. In our cross-foster study of PFOA with CD-1 mice, control pups fostered to treated dams survived and did not have weight deficits, but pups exposed *in utero* and fostered to control dams exhibited a high incidence of mortality and surviving pups had low weights relative to control pups, suggesting that neither maternal behaviors nor lactational insufficiency were involved, and that *in utero* exposure was required to produce the postnatal effects [33].

The mechanism(s) through which PFOS and PFOA produce developmental toxicity remains unclear, but activation of peroxisome proliferator activated receptor-alpha (PPARα) may be a factor, as PFOS and PFOA are weak activators of the receptor and both induce hepatic peroxisome proliferation. PPARα is a ligand-activated nuclear receptor that regulates gene expression in a characteristic manner and has functions related to cell proliferation and differentiation [34,35]. PPARα is present during embryonic development and its expression varies with developmental stage but PPARα mRNA and/or protein was found in the gastrointestinal tract, liver, kidney, heart, fat, central nervous system, vertebrae, skin, muscle, and lung [36–42]. The PPARα knockout (PPAR KO) mouse was used in our previous studies [43] to evaluate whether the developmental toxicity of PFOA is mediated through the PPARα pathway. In these studies, PFOA-induced postnatal lethality in wild type (WT) and heterozygous pups (even when heterozygotes were born to PPAR KO dams), but not in PPAR KO pups. Delayed eye opening and deficits in postnatal weight gain also appeared to depend on PPARα expression, although other mechanisms may contribute to producing those effects. It was concluded that PPARα was required for PFOA to produce the postnatal lethality and that expression of only one copy of the gene was sufficient.

Both PFOA and PFOS are peroxisome proliferators and weak agonists for PPARα, and the developmental toxicities of these compounds are very similar in the mouse. Since PPARα is involved in PFOA-induced postnatal lethality in mice, the present study was undertaken to determine if postnatal lethality observed in neonates exposed to PFOS during gestation is also mediated by the PPARα pathway. This study used PPARα knockout and 129S1 wild type mice to examine the survival, weight gain and eye opening of pups exposed during gestation to PFOS. It is concluded that PFOS-induced neonatal lethality and delayed eye opening are not dependent on activation of PPARα and that the mechanisms leading to neonatal lethality differ for PFOS (PPARα-independent) and PFOA (PPARα-dependent).

2. Materials and methods

2.1. Animals

Male and female 129S1/SvlmJ wild type and PPARα knockout mice were originally obtained from Jackson Laboratories (Bar Harbor, MA), and a breeding colony was established at EPA. The mice were housed in ventilated Tecniplast cages (Tecniplast USA, Exton, PA) and provided pellet chow (LabDiet 5001, PMI Nutrition International, Brentwood, MO) and tap water ad libitum. Animal facilities were controlled for temperature (20–24 °C) and relative humidity (40–60%), and kept under a 12-h light–dark cycle. All animal studies were conducted in accordance with guidelines established by the US EPA ORD/NHEERL Institutional Animal Care and Use Committee. Procedures and facilities were consistent with the recommendations of the 1996 NRC "Guide for the Care and Use of Laboratory Animals", the Animal Welfare Act, and Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

2.2. Study protocol

Perfluorooctane sulfonate (potassium salt; >91% pure) was purchased from Fluka Chemical (Steinhiem, Switzerland). PFOS was dissolved in 0.5% Tween-20 in water and all dosing solutions were prepared fresh daily. Male and female mice of the same strain were bred overnight and the next morning females were examined for presence of a mating plug and this was designated GD0. On GD15, pregnant mice were weighed and randomly assigned to treatment groups. On GD15–18, mice were weighed daily and dosed by gavage with either vehicle (0.5% Tween-20) or PFOS. WT mice were dosed at 4.5, 6.5, 8.5, or 10.5 mg/kg/day (10 mL/kg) and KO mice were dosed with vehicle or PFOS at 8.5 or 10.5 mg/kg/day.

Selection of the dosing period during gestation was based on the effects of PFOS on postnatal survival in the Sprague–Dawley rat. Grasty et al. [44] reported that exposure during the final 4 days of gestation was sufficient to cause postnatal death. These studies observed similar outcomes whether the dose was administered as 5 mg/kg/day for 20 days (GD2–21) or as 25 mg/kg/day across 4 days (GD17–20) and in each regimen the same total dose was administered (100 mg/kg). Thus, in the present study, the WT and KO mice were exposed to PFOS only on the last 4 days of gestation.

The selection of the dose range for PFOS in the present study was influenced by a difference in sensitivity between CD-1 and WT mice in their response to PFOA. WT mice (which are on a 129S1/SvlmJ genetic background), were observed to be about 8–10 times more sensitive than CD1 mice to the effects of PFOA on neonatal survival [43], and unpublished observations. It was anticipated that there could be a similar strain difference in sensitivity to PFOS. Lau et al. [25] reported a significant reduction in postnatal survival in CD-1 mice exposed to PFOS after dosing from GD1 to 17 at 15 and 20 mg/kg/day, total doses of 260 and 340 mg/kg, respectively. In the present study, mice with a 129S genetic background received one tenth of that total dose (26 and 34 mg/kg) across the last 4 days of gestation, i.e. 6.5 and 8.5 mg/kg/day. A lower and higher dose (4.5 and 10.5 mg/kg/day) were then selected to complete the dose range.

At parturition (PND1), the number of live and dead pups was recorded and the live pups in each litter were weighed as a group. The number of live pups in each litter was recorded daily on PND1–15. Eye opening was monitored from PND 12 to 15. Litter weights were recorded by gender at PND15 and the pups and dams were euthanized at that time. (*Note*: The study was conducted in three blocks and data for the maternal weights, reproductive outcomes, serum levels, and pup survival were collected from all three blocks. Pup weight, eye opening, pup liver weight, and pup serum level data were collected only from blocks 2 and 3.) Adult females were weighed, livers removed and weighed, and uteri stained with 2% ammonium sulfide to count implantation sites [45]. Percent litter loss from implantation to birth was calculated as (#implantation sites − #live pups at birth)/(#implantation sites) × 100. Two pups from each litter (where possible, one of each sex), were weighed individually and liver weight recorded. Blood was collected from adult females and pups (pooled from all pups in the litter) and serum prepared and stored frozen at −80 °C for later PFOS analysis.

2.3. Serum PFOS determination

Analysis of PFOS in serum was performed using a modification of a method originally developed by Hansen et al. [46]. Briefly, 25 μL of serum was combined with

Table 1
Maternal weights and reproductive outcomes in wild type and PPARα knockout (PPAR KO) mice after exposure to perfluorooctane sulfonate (PFOS) on GD15–18

Dose (mg/kg/day)	Number of dams	Maternal weight (g) GD18	Maternal weight gain (g) GD15–18	Implants per dam	Total number of pups per litter[a]	%Litter loss[b]
Wild type						
0	20	36.6 ± 0.7	3.8 ± 0.4	9.1 ± 0.4	6.5 ± 0.4	41.2 ± 6.7
4.5	8	37.3 ± 1.1	4.4 ± 0.7	9.1 ± 0.7	6.8 ± 0.8	30.0 ± 5.2
6.5	13	33.8 ± 1.2	2.2 ± 0.8	8.1 ± 0.5	5.8 ± 0.8	48.4 ± 11.4
8.5	22	37.2 ± 0.6	4.0 ± 0.3	9.0 ± 0.5	7.0 ± 0.5	24.1 ± 4.9
10.5	17	37.0 ± 0.8	3.8 ± 0.3	9.4 ± 0.5	6.9 ± 0.4	30.6 ± 4.1
PPAR KO						
0	13	36.3 ± 0.7	4.0 ± 0.4	8.5 ± 0.5	6.9 ± 0.6	24.3 ± 7.3
8.5	16	34.5 ± 1.1	2.4 ± 0.6	8.8 ± 0.5	5.4 ± 0.7	42.4 ± 6.0
10.5	14	37.3 ± 1.0	3.9 ± 0.3	10.1 ± 0.5	7.1 ± 0.5	29.0 ± 4.7

Means (dams) or litter means (pups) ± S.E.M. GD, gestational day.
[a] Total number of live + dead at birth.
[b] Percent litter loss from implantation to birth = (#implants − #live pups at birth)/#implants × 100.

1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (pH 10) and 2 mL of 0.25 M sodium carbonate in a 15 mL polypropylene tube and then vortexed for 20 min. Three hundred microliters of this mixture was then transferred to a fresh 15 mL polypropylene tube and 25 μL of a 1 ng/μL solution of $^{18}O_2$-PFOS (RTI International, Research Triangle Park, NC) was added as an internal standard. Five milliliters of methyl *tert*-butyl ether (MTBE) was then added and vortexed again for 20 min. The tube was centrifuged to separate the aqueous and organic phases, and 1 mL of the MTBE layer was transferred to a fresh 15 mL polypropylene tube, combined with 0.5 mL of acetonitrile, and concentrated to ≤200 μL at 55 °C using a TurboVap (Caliper Life Sciences, Hopkinton, MA). The residue was mixed with 200 μL of 2 mM ammonium acetate and transferred to a polypropylene autosampler vial. Extracts were analyzed using an Agilent 1100 high-performance liquid chromatograph (Agilent Technology, Palo Alto, CA) coupled with an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) (LC/MS/MS). Ten microliters of the extract was injected onto a Luna C18(2) 3 mm × 50 mm, 5 μm column (Phenomenex, Torrance, CA) using an isocratic mobile phase consisting of 30% 2 mM ammonium acetate solution and 70% acetonitrile at a flow rate of 200 μL/min. PFOS and $^{18}O_2$-PFOS were monitored using parent and daughter ion transitions of 499.0 > 80.0 and 503.0 > 83.9, respectively. Peak integrations and areas were determined using Analyst Software (Applied Biosystems Version 1.4.1). For each analytical batch, matrix-matched calibration curves were prepared using mouse serum spiked with varying levels of PFOS (Fluka Chemical, Steinheim, Switzerland) as described above. For quality control (QC), check standards were prepared by spiking large volumes of mouse serum at several arbitrary levels. These check standards were stored frozen and aliquots analyzed with each analytical set. In addition, control mouse serum samples were fortified at two or three levels in duplicate with known quantities of PFOS during the preparation of each analytical set. Duplicate fortified and several check standards were run in each analytical batch to assess precision and accuracy. The limit of quantitation (LOQ) was set as the lowest calibration point on the standard curve. Analytical batches were considered to be acceptable if: matrix and reagent blanks had no significant PFOS peaks approaching the LOQ, the standard curve had a correlation coefficient >0.98, and all standard curve points, fortified and check samples were within 70–130% of the theoretical and previously determined values, respectively.

2.4. Statistical analysis

Statistical analyses were run using Prism 4.0, GraphPad Software (San Diego, CA). Means and standard errors of the mean (S.E.M.) were calculated for each group and differences between groups were determined using analyses of variance (ANOVA) followed by pairwise tests with adjustments for multiple comparisons using Dunnett's test. Linear regression models were also run to test for trends across dose for PND15 pup liver weight, relative liver weight, and serum PFOS levels. Analyses were performed separately by strain (KO and WT), and pup data were analyzed on a litter basis. PND15 pup weight, liver weight and liver/body weight ratios were analyzed separately for male and female, as well as after combining the data for both sexes.

Means and standard errors of serum PFOS levels were calculated by SAS Proc Means and tests of differences were performed on square root transformed data sets using ANOVA models by Proc Means and Proc Mixed and, as needed to adjust for the relationship of dam and pup values, a random effect for dam was included (SAS/STAT User's Guide, Version 9, Cary, NC, SAS Institute Inc., 2003). For some control samples, the level of PFOS was below the LOQ and for the analysis and to calculate mean and standard error, the LOQ value (either 5 or 50 ng/mL, depending on the standard curve for the assay) was substituted. Comparison of treatment groups and controls used Dunnet's test with adjustments for multiple comparisons.

3. Results

Maternal weight on GD18 and weight gain from GD15 to 18 were unaffected by PFOS exposure at any dose in both WT and KO dams (Table 1). The number of embryonic implantation sites, the total number of pups at birth (live and dead pups) and the percent litter loss from implantation to birth were not significantly affected by PFOS exposure in either WT or KO (Table 1). Pup birth weight, pup weight on PND15, and weight gain from PND1 to 15 were not significantly affected by PFOS in either WT or KO litters (Table 2). PND15 pup weights and weight gain shown in Table 2 are based on combining data for male and female pups in a litter. Pup weight of PND15 males and females was not differentially affected by PFOS exposure (data not shown).

PFOS exposure did not affect body weight, absolute or relative liver weight in either the WT or KO dams (body and liver

Table 2
Birth weight and weight gain PND1–15 in wild type and PPARα knockout (PPAR KO) mice after exposure to perfluorooctane sulfonate (PFOS) on GD15–18

Dose (mg/kg/day)	Number of litters	Live pup birth weight[a] (g)	Pup weight PND15[b] (g)	Weight gain PND1–15[b] (g)
Wild type				
0	13	1.38 ± 0.04	7.65 ± 0.19	6.30 ± 0.19
4.5	8	1.40 ± 0.05	7.67 ± 0.47	6.33 ± 0.45
6.5	4	1.32 ± 0.03	7.91 ± 0.27	6.59 ± 0.29
8.5	15	1.35 ± 0.04	7.16 ± 0.27	5.83 ± 0.27
10.5	17	1.39 ± 0.02	7.49 ± 0.67	6.14 ± 0.67
PPAR KO				
0	11	1.30 ± 0.04	7.03 ± 0.33	5.76 ± 0.34
8.5	11	1.27 ± 0.03	7.31 ± 0.30	6.05 ± 0.31
10.5	14	1.25 ± 0.04	6.25 ± 0.43	4.99 ± 0.43

Litter means ± S.E.M. GD, gestational day; PND, postnatal day.
[a] Male and female pups in a litter weighted as a group on PND1.
[b] Male and female pups were weighed by sex on PND15. Mean PND15 weight and weight gain are based on combining data for males and females by litter.

Table 3
Body weight, absolute, and relative liver weight in dams and offspring on PND15 in wild type and PPARα knockout (PPAR KO) mice after exposure to perfluorooctane sulfonate (PFOS) on GD15–18

Dose group	*N*	Body weight (g)	Liver weight (g)	Relative liver weight[a]
WT dams				
0	20	28.7 ± 0.72	1.63 ± 0.10	5.61 ± 0.23
4.5	8	28.6 ± 1.23	1.74 ± 0.13	6.02 ± 0.22
6.5	11	26.7 ± 1.06	1.66 ± 0.12	6.15 ± 0.21
8.5	22	27.8 ± 0.66	1.71 ± 0.07	6.11 ± 0.13
10.5	17	27.3 ± 0.88	1.72 ± 0.10	6.22 ± 0.16
KO dams				
0	13	29.9 ± 1.09	1.89 ± 0.14	6.23 ± 0.32
8.5	16	28.6 ± 0.99	1.81 ± 0.12	6.24 ± 0.24
10.5	14	30.6 ± 0.90	2.18 ± 0.10	7.08 ± 0.15
WT pups				
0	9	7.59 ± 0.20	0.24 ± 0.01	3.11 ± 0.54
4.5	6	7.72 ± 0.43	0.26 ± 0.02	3.33 ± 0.12
6.5	4	7.91 ± 0.35	0.27 ± 0.01	3.43 ± 0.13
8.5	9	6.95 ± 0.46	0.28 ± 0.01	3.52 ± 0.22
10.5	7	7.43 ± 0.66	0.30 ± 0.03[b]	4.00 ± 0.19[c]
KO Pups				
0	10	7.30 ± 0.30	0.23 ± 0.01	3.18 ± 0.06
8.5	7	7.41 ± 0.28	0.25 ± 0.01	3.48 ± 0.10
10.5	13	6.28 ± 0.51	0.23 ± 0.02	3.70 ± 0.15[d,b]

Means (dams) or litter means (pups) ± S.E.M. *N*, number of dams or litters (pup means on a litter basis). Liver and body weights shown here are from two pups per litter (generally one of each sex).

[a] Relative liver weight = (liver weight/body weight) × 100.

[b] $p < 0.01$, trend for increase across dose.

[c] $p < 0.001$, treated vs. control and trend for increase across dose.

[d] $p < 0.05$, treated vs. control.

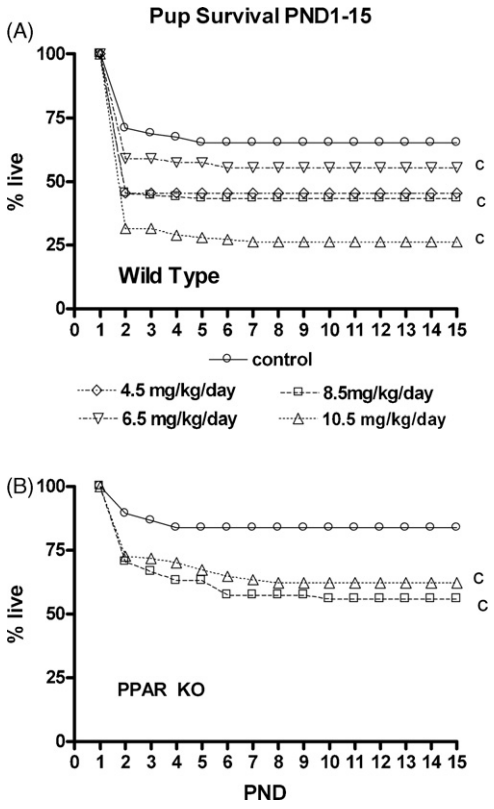

Fig. 1. Postnatal survival of pups is shown as percent of the litter alive on PND1–15 for WT (A) and PPAR KO (B) strains. WT litters showed a significant decrease in survival to PND15 in the 4.5, 8.5, and 10.5 mg/kg/day groups. KO litters had significantly decreased survival to PND15 in the 8.5 and 10.5 mg/kg/day groups, compared to the KO control. Data shown are the mean of all litters in each dose group. [c] $p < 0.001$ vs. control.

weights measured on PND15 at 16 days after the last dose) at any of the doses administered (Table 3). PFOS exposure did not affect the mean pup body weight on PND15, however, relative liver weights of WT and KO pups exposed on GD15–18 to PFOS at 10.5 mg/kg were significantly increased (sexes combined). There were also significant dose-related trends for increased relative liver weight in both WT and KO pups and for increased absolute liver weight in the WT pups. (*Note*: PND15 mean pup weights shown in Table 2 were calculated from the average weight of all pups in each litter, while the liver and body weights shown in Table 3 are from two pups per litter, generally one of each sex.) When relative liver weight of pups was examined separately by sex, a significant effect of PFOS was detected in WT males and females ($p < 0.05$) exposed to 8.5 mg/kg/day, as well as WT males ($p < 0.01$), WT females ($p < 0.001$), and KO males ($p < 0.05$) exposed to the 10.5 mg/kg/day dose (data not shown). In addition, absolute pup liver weight of the 10.5 mg/kg/day dose group were significantly increased in WT males and WT females ($p < 0.05$ and <0.01, respectively, data not shown).

Survival of pups from birth to PND15 was significantly reduced in PFOS-exposed WT and KO litters (Fig. 1A and B). Most of the postnatal deaths occurred between PND1 and 2. Survival of WT pups on PND15 was significantly reduced ($p < 0.001$) in the 4.5, 8.5, and 10.5 mg/kg/day groups, compared to WT controls. WT litters exposed to 4.5, 6.5, 8.5, and 10.5 mg/kg/day had only 45 ± 14% ($n = 8$), 55% ± 6 ($n = 7$), 43% ± 9 ($n = 20$), or 26 ± 9% ($n = 17$) of pups alive on PND15, respectively, compared to 65 ± 10% ($n = 16$) in control litters. In WT pups, survival in the 4.5 mg/kg/day group did not significantly differ from that of pups exposed to 6.5 or 8.5 mg/kg/day. Survival of KO pups on PND15 was significantly reduced ($p < 0.001$) in the 8.5 and 10.5 mg/kg/day groups, compared to KO controls. KO litters exposed to 8.5 or 10.5 mg/kg/day had 56% ± 12 ($n = 13$) or 62 ± 8% ($n = 14$) of pups alive on PND15, compared to 84 ± 9% ($n = 12$) in control litters. The effects of PFOS on survival could be expressed as % of the respective controls to take into account the different rates of survival that may be related to strain. Even though the genetic background of both the WT and KO mice was 129S1, the survival of WT controls was lower than that of the KO. If survival of the PFOS-exposed pups is expressed as % of control, the survival in the 8.5 mg/kg dose groups is the same regardless of strain (66.4% in WT and 66.6% in KO). The effect of the 10.5 mg/kg dose was more pronounced in the WT, even when expressed as % of control (40.2% survival in WT and 72.4% in KO).

Postnatal development was also evaluated by recording the age at which both eyes were fully opened and calculating percent of pups per litter with open eyes. The percent of pups per litter with both eyes open increased from PND12 to 15 for all groups, WT and KO (Fig. 2A and B). On PND13, none of the WT pups exposed to 8.5 mg/kg/day ($n = 8$ litters) had open eyes, while 44% ± 15 of the controls ($n = 9$ litters) had open eyes ($p < 0.05$ compared to control on that day). On PND14, the percent of KO pups with both eyes open was significantly ($p < 0.05$) lower in the group exposed to 10.5 mg/kg/day (23% ± 10, $n = 13$), compared to the KO controls (59% ± 10, $n = 10$). These results suggest that eye opening was delayed by exposure to PFOS in both the WT and KO pups.

Serum levels of PFOS in dam and pups were determined from samples collected on PND15 (16 days after the last dose). The level of PFOS in serum of adult females and PND15 pups increased with dose in a linear fashion (significant dose-related trend, $p < 0.001$, r^2 for the groups ranged from 0.78 to 0.95) in both WT and KO mice (Table 4). All treatment groups were significantly different from their respective controls ($p < 0.001$). Under the assay conditions employed here, PFOS was below the limits of detection in

Table 4
Serum levels of PFOS in adult females and pups on PND15

Dose group	Adult females: no pups at PND15[a]		Adult females: with pups at PND15[b]		Pup at PND15	
	N	PFOS (ng/mL)	*N*	PFOS (ng/mL)	*N*	PFOS (ng/mL)
Wild type						
0	7	15.8 ± 4.73	13	17.5 ± 6.27	8	7.39 ± 2.92
4.5	2	32,400 ± 2650	6	12,500 ± 3230	6	24,100 ± 1820
6.5	4	41,600 ± 2950	7	16,300 ± 1780	4	28,700 ± 2610
8.5	10	45,000 ± 3150	12	15,200 ± 2040	8	40,700 ± 2680
10.5	10	57,700 ± 3780	7	22,600 ± 3570	6	41,200 ± 3070
PPARα KO						
0	2	42.5 ± 7.55	9	58.2 ± 8.22	8	6.88 ± 1.57
8.5	6	56,400 ± 5460	9	15,700 ± 2230	7	42,800 ± 3600
10.5	1	73,800	13	26,600 ± 3410	12	52,400 ± 3620

Means ± S.E.M. Data were rounded to three significant figures. WT control dams with pups, 7 had values <LOQ; KO dams without or with pups, 1 and 8 had values <LOQ, respectively. LOQ value was used for these dams and as a consequence the calculated mean and S.E.M. are likely to be overestimations for these groups.

[a] Adult females dosed from GD15 to 18, with no pups surviving past PND6.

[b] Adult females dosed GD15–18 with live pups at PND15.

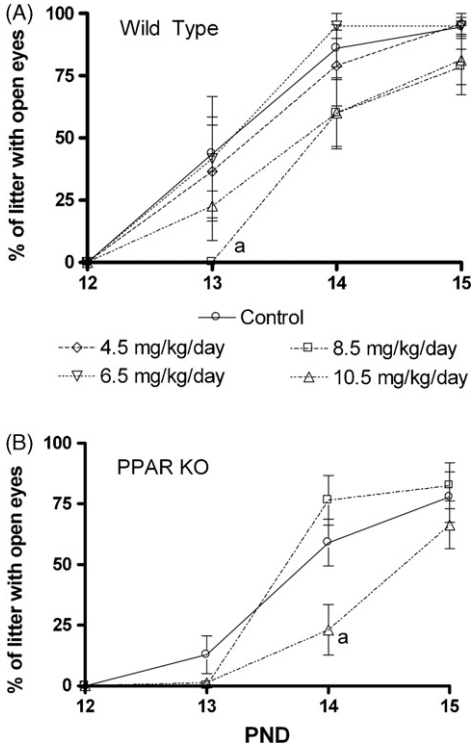

Fig. 2. Percentage of pups with both eyes fully open on PND12–15 is shown for WT (A) and PPAR KO (B) litters. Eye opening was delayed in WT litters exposed to 8.5 mg/kg/day and PPAR KO litters exposed to 10.5 mg/kg/day, [a] $p < 0.05$ vs. control.

most control samples; therefore, the means reported for the control groups shown in Table 4 reflect the LOQ values (5 or 50 ng/mL) used for the statistical analyses. WT and KO mice receiving the same dose had comparable PFOS levels on PND15, with the exception of the dams with no pups which received the 8.5 mg/kg dose and the pups from dams dosed at 10.5 mg/kg (KO > WT, $p < 0.05$). The level of PFOS in the pups was significantly higher than in the PFOS-treated dams ($p < 0.001$) (comparing the levels in PFOS-exposed dams to the levels in the pups). Dams without pups after PND6 had significantly higher levels of PFOS than the dams with surviving pups ($p < 0.001$).

4. Discussion

In utero exposure of the mouse to either PFOA or PFOS can result in high incidences of neonatal death. Although the processes that are involved in producing deaths of the newborn mice remain unclear, the responses to PFOA and PFOS differ regarding the requirement for the PPARα pathway. The present study showed that exposure to PFOS during the last 4 days of gestation results in neonatal death soon after birth and that this response occurs in the absence of expression of PPARα. Thus, activation of PPARα is not required in order to produce neonatal deaths after gestational exposure to PFOS. This is not the case for PFOA, as our earlier study demonstrated that expression of PPARα is required to mediate the responses that lead to neonatal death [43]. The PFOA-exposed mice that did not express PPARα survived, while heterozygous or wild type mice died. Thus, although PFOS and PFOA are both capable of producing neonatal death, it seems likely that there is more than one mechanism involved in producing that response.

The developmental toxicity of PFOS and PFOA in rat and mouse were initially examined using protocols that dosed throughout gestation. These studies found dose-related effects on postnatal survival, as well as effects on weight gain and delays in eye opening [25–28,31,47]. Cross-foster studies demonstrated that these effects were dependent on gestational exposure and eliminated maternal behavior or lactational exposure as major contributors to the outcomes [25,33]. Further studies examined the sensitive developmental period for producing these effects and showed that exposure on the last few days of gestation was sufficient to produce the neonatal lethality. Wolf et al. [33] reported that exposure of CD-1 mice to PFOA at 5 mg/kg/day on GD15–17 was sufficient to reduce pup weight from birth to PND22 and delay eye opening. Dosing CD-1 mice with PFOA from GD15 to 17 was also able to reduce neonatal survival, but only at the high dose of 20 mg/kg/day. These data indicate that PFOA exposure only on the last few days of gestation is sufficient to produce neonatal lethality in mice. Similarly, Grasty et al. [44] dosed Sprague–Dawley rats with PFOS at 25 mg/kg/day for 4 days at different stages of pregnancy and found that exposure late in gestation would result in neonatal deaths. In fact, one group of rats in that study was exposed to PFOS on GD19–20 at 50 mg/kg/day and only 29% of the pups born to these dams survived to 12 h after birth.

The identification of a critical period late in gestation for the effects of PFOS that lead to neonatal death suggests that an organ such as the lung, that matures morphologically and functionally late in gestation may be a target. Studies using rat pups exposed to PFOS at 10 mg/kg/day from GD2 to 21 or mouse pups exposed to PFOS at 15 mg/kg/day from GD1 to 17, reported that breathing was labored and pups became pale, inactive and moribund within 30–60 min after birth [25]. Grasty et al. [44,48] examined the lungs of newborn rats exposed to PFOS and described histological and morphometric effects that suggested effects on maturation of the

organ. For example, the alveolar epithelium retained an appearance similar to the prenatal lung (thicker alveolar walls with increased tissue to air space ratios). Lungs did not appear to expand fully upon perfusion with fixative into the airspaces. The possibility that PFOS was affecting the production or composition of lung surfactant was investigated [48]. Lung surfactant is produced late in gestation and its major function is to reduce the surface tension at the air–water interface in terminal airways and prevent collapse of the alveolus upon expiration. Lung surfactant forms a surface-active film that is composed of approximately 80% phospholipids, 10% neutral lipids, and 10% proteins [49]. Grasty et al. [48] examined the phospholipid profile and molecular composition of total phosphotidylcholines present in control and PFOS-exposed neonatal rat lung and amniotic fluid on GD21 but found no significant changes. Gene array analysis of the lungs also failed to identify any changes in gene expression patterns that would indicate effects on surfactant production or alveolar epithelial differentiation. While these studies in the rat implicated lung function in the neonates as a possible cause of the early deaths observed in PFOS-exposed pups, it remains unclear if the mechanism involves lung maturation *per se* or changes in the production or composition of lung surfactant. Although the degree of lung development at birth varies between species, alveoli increase in number and surface area postnatally in both humans and rodents [50]. Although Gratsy did not detect effects of PFOS on surfactant composition or production on GD21 in the rat, there is a possibility that effects on the lung could occur after birth and impact the progressive development of alveoli and acquisition of lung function during the postnatal period.

Perfluorinated compounds have chemical properties that make them excellent surfactants and there is a possibility that PFOS or PFOA could directly interact with pulmonary surfactant and interfere with its function. Several recent studies have evaluated the potential for PFOS and PFOA to interact with the main components of lung surfactant. Dipalmitoylphosphatidylcholine (DPPC) is one of the main phospholipid components in surfactant. Lehmler et al. [51] used DPPC model bilayer membranes to evaluate the effects of PFOS on membrane stability using differential scanning calorimetry and fluorescence anisotropy measurements. PFOS was found to partition into and affect the model membranes, changing membrane fluidity at concentrations as low as 10 mg/L. The apparent surfactant-DPPC partition coefficients for PFOS and sodium perfluorooctanoate were found to be 5.7×10^4 and 8.9×10^3, respectively. These investigators recently compared the behaviors of the potassium salt of PFOS, PFOA, and octane sulfonate (OS) with model DPPC bilayer membranes [52]. The apparent partition coefficients of PFOS, PFOA, and OS between DPPC bilayers and the aqueous phase were 5.6×10^4, 1.5×10^4, and 3.0×10^2, respectively. The partition coefficient for PFOS was about four times larger than that for PFOA. Both PFOS and PFOA were shown to partition into the DPPC bilayer and alter membrane properties at relatively low concentrations. PFOS altered T_m, the temperature at the midpoint of the liquid-crystalline to the gel phase transition, at concentrations as low as 19 μmol/L (~10 ppm), while PFOA affected phase behavior with a significant decrease in T_m at 111 μmol/L (~48 ppm). These studies demonstrated that PFOS and PFOA have a tendency to partition into DPPC model membranes, alter membrane structure, and possibly membrane function, but PFOS does so more drastically and at lower concentrations. Similar findings were recently reported using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) monolayer and bilayer model membranes and NMR techniques, the Langmuir–Blodgett technique with surface pressure and surface potential measurements, and molecular dynamics simulations to compare PFOS and PFOA [53]. These studies showed that PFOS and PFOA migrate from the water phase into the preformed DPPC and DMPC monolayers and bilayers, changing their properties. Both PFOS and PFOA influenced the fluidity and phase transitions of the DPPC and DMPC membranes, but PFOS produced stronger effects. Studies using a biological surfactant preparation, bovine lipid extract surfactant and a captive bubble surfactometer also support the ability of PFOS and PFOA to directly interfere with surfactant properties at relatively low concentrations [54].

These *in vitro* studies support the hypothesis that perfluorinated chemicals have the potential to directly interact with lung surfactant and that disruption of the function of lung surfactant could be responsible for the neonatal mortality observed in rodent studies. While this hypothesized mechanism might be applicable to those pups that exhibit labored breathing and die soon after birth, this mode of action is less likely to explain the deaths that occur later in the postnatal period because pups exposed to PFOS at low doses or to PFOA continue to die for up to 10–14 days after birth. It is likely that other organs and/or physiological processes are targeted by these compounds to produce the later deaths. Based on the cross-foster studies [25,33], it was apparent that prenatal exposure to PFOS was an important determinant of the outcomes, and an attempt to induce the neonatal lethality by directly treating newborn pups did not lead to death (unpublished data, C. Lau). In addition to distribution of the compounds to the fetal tissues, amniotic fluid was proposed as a route of exposure that would give the compound direct access to the alveoli and surfactant in the fetal and newborn lung. PFOA has been detected in amniotic fluid on GD15 and 21 in the rat with dose dependent concentrations ranging from 1.5 to 8.1 μg/mL [30] and in their study Grasty et al. [48] also detected PFOS in rat amniotic fluid (personal communication, J. Rogers).

The present study showed that PFOS-induced neonatal deaths regardless of the status of PPARα gene expression, an observation that is consistent with a mechanism that depends on the chemical properties of the compound and not on altered gene expression as a primary event. The finding that PFOS is more effective than PFOA in changing the properties of phospholipid membranes and the fact that PFOS is a weaker agonist for PPARα than PFOA [55–57], may help explain the apparent difference in mechanisms through which PFOA and PFOS cause neonatal deaths. Thus, the mode of action for PFOS-induced neonatal lethality found to occur soon after birth could involve interference with the function of lung surfactant, but may not require activation of the PPARα receptor. PFOA, on the other hand, is a better agonist for PPARα than PFOS and is weaker in its ability to disrupt phospholipid bilayers. Furthermore, neonatal lethality after PFOA exposure is dependent on expression of PPARα. This study, along with those which examined the potential for PFOS and PFOA to interfere with lipid bilayers, only provide indirect evidence to suggest pulmonary insufficiency as a cause of PFOS-induced neonatal lethality. In addition, the role of PPARα activation in postnatal deaths remains to be elucidated. Clearly, additional studies will be needed to definitively define the modes of action for the deaths that occur soon after birth and to establish causes for deaths that occur days later.

Acknowledgements

The authors gratefully acknowledge Laurence Helfant and Shoji Nakayama, Human Exposure and Atmospheric Sciences Division, National Exposure Research Laboratory, EPA, for assistance in the analysis of the PFOS in serum samples. The investigators relied on Mr. David Kuhn, SEE employee, RTD, NHEERL, EPA for his assistance in the management and record keeping for the wild type and PPARα KO animal colonies. The authors also appreciate the contributions

of Ms. Vivian Williams, SEE employee, RTD, NHEERL, EPA, who participated with Mr. Kuhn in assuring the accuracy of data entry for the study.

References

[1] Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environ Sci Technol 2002;36(15):3210–6.

[2] Kannan K, Tao L, Sinclair E, Pastva SD, Jude DJ, Giesy JP. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch Environ Contam Toxicol 2005;48(4):559–66.

[3] Loganathan BG, Sajwan KS, Sinclair E, Senthil Kumar K, Kannan K. Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Res 2007.

[4] Loos R, Locoro G, Huber T, Wollgast J, Christoph EH, de Jager A, et al. Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy. Chemosphere 2007.

[5] Yamashita N, Taniyasu S, Petrick G, Wei S, Gamo T, Lam PK, et al. Perfluorinated acids as novel chemical tracers of global circulation of ocean waters. Chemosphere 2007.

[6] Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. Serum concentrations of 11 polyfluoroalkyl compounds in the U.S. population: data from the national health and nutrition examination survey (NHANES). Environ Sci Technol 2007;41(7):2237–42.

[7] Renner R. Growing concern over perfluorinated chemicals. Environ Sci Technol 2001;35:154A–60A.

[8] Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicol Sci 2002;68(1):249–64.

[9] Kissa E. Fluorinated Surfactant and Repellants. New York: Marcel Decker; 2001.

[10] Giesy JP, Kannan K. Perfluorochemical surfactants in the environment. Environ Sci Technol 2002;36(7):146A–52A.

[11] Hansen KJ, Johnson HO, Eldridge JS, Butenhoff JL, Dick LA. Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. Environ Sci Technol 2002;36(8):1681–5.

[12] Hoff PT, Van de Vijver K, Van Dongen W, Esmans EL, Blust R, De Coen WM. Perfluorooctane sulfonic acid in bib (*Trisopterus luscus*) and plaice (*Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea: distribution and biochemical effects. Environ Toxicol Chem 2003;22(3):608–14.

[13] Kubwabo C, Vais N, Benoit FM. A pilot study on the determination of perfluorooctanesulfonate and other perfluorinated compounds in blood of Canadians. J Environ Monit 2004;6(6):540–5.

[14] 3MCompany. Environmental and health assessment of perfluorooctanesulfonate and its salts. US EPA Administrative Record; 2003.

[15] Olsen GW, Mair DC, Reagen WK, Ellefson ME, Ehresman DJ, Butenhoff JL, et al. Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations in American Red Cross blood donors. Chemosphere 2007;68(1):105–11.

[16] Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. Environ Health Perspect 2007;115(11):1596–602.

[17] Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH. Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. Environ Sci Technol 2003;37(5):888–91.

[18] Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, et al. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ Health Perspect 2003;111(16):1892–901.

[19] Olsen GW, Church TR, Larson EB, van Belle G, Lundberg JK, Hansen KJ, et al. Serum concentrations of perfluorooctanesulfonate and other fluorochemicals in an elderly population from Seattle, Washington. Chemosphere 2004;54(11):1599–611.

[20] Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect 2007;115(11):1670–6.

[21] Volkel W, Genzel-Boroviczeny O, Demmelmair H, Gebauer C, Koletzko B, Twardella D, et al. Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk: results of a pilot study. Int J Hyg Environ Health 2007.

[22] Harada K, Saito N, Inoue K, Yoshinaga T, Watanabe T, Sasaki S, et al. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. J Occup Health 2004;46(2):141–7.

[23] Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 2007;99(2):366–94.

[24] Kennedy Jr GL, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, et al. The toxicology of perfluorooctanoate. Crit Rev Toxicol 2004;34(4):351–84.

[25] Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II. Postnatal evaluation. Toxicol Sci 2003;74(2):382–92.

[26] Luebker DJ, Case MT, York RG, Moore JA, Hansen KJ, Butenhoff JL. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 2005;215(1/2):126–48.

[27] Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague–Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology 2005;215(1/2):149–69.

[28] Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I. Maternal and prenatal evaluations. Toxicol Sci 2003;74(2):369–81.

[29] Butenhoff JL, Kennedy Jr GL, Frame SR, O'Connor JC, York RG. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 2004;196(1/2):95–116.

[30] Hinderliter PM, Mylchreest E, Gannon SA, Butenhoff JL, Kennedy Jr GL. Perfluorooctanoate: placental and lactational transport pharmacokinetics in rats. Toxicology 2005;211(1/2):139–48.

[31] Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, et al. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci 2006;90(2):510–8.

[32] Case MT, York RG, Christian MS. Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. Int J Toxicol 2001;20(2):101–9.

[33] Wolf CJ, Fenton SE, Schmid JE, Calafat AM, Kuklenyik Z, Bryant XA, et al. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicol Sci 2007;95(2):462–73.

[34] Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20(5):649–88.

[35] Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. Cell Mol Life Sci 2004;61(4):393–416.

[36] Beck F, Plummer S, Senior PV, Byrne S, Green S, Brammar WJ. The ontogeny of peroxisome-proliferator-activated receptor gene expression in the mouse and rat. Proc Biol Sci 1992;247(1319):83–7.

[37] Braissant O, Wahli W. Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. Endocrinology 1998;139(6):2748–54.

[38] Balasubramaniyan N, Shahid M, Suchy FJ, Ananthanarayanan M. Multiple mechanisms of ontogenic regulation of nuclear receptors during rat liver development. Am J Physiol Gastrointest Liver Physiol 2005;288(2):G251–260.

[39] Huin C, Corriveau L, Bianchi A, Keller JM, Collet P, Kremarik-Bouillaud P, et al. Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract. J Histochem Cytochem 2000;48(5):603–11.

[40] Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, et al. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci USA 1994;91(15):7355–9.

[41] Keller JM, Collet P, Bianchi A, Huin C, Bouillaud-Kremarik P, Becuwe P, et al. Implications of peroxisome proliferator-activated receptors (PPARS) in development, cell life status and disease. Int J Dev Biol 2000;44(5):429–42.

[42] Panadero M, Herrera E, Bocos C. Peroxisome proliferator-activated receptor-alpha expression in rat liver during postnatal development. Biochimie 2000;82(8):723–6.

[43] Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, et al. Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. Toxicol Sci 2007;98(2):571–81.

[44] Grasty RC, Grey BE, Lau CS, Rogers JM. Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague–Dawley rat. Birth Defects Res B Dev Reprod Toxicol 2003;68(6):465–71.

[45] Narotsky MG, Brownie CF, Kavlock RJ. Critical period of carbon tetrachloride-induced pregnancy loss in Fischer-344 rats, with insights into the detection of resorption sites by ammonium sulfide staining. Teratology 1997;56(4):252–61.

[46] Hansen KJ, Clemen LA, Ellefson ME, Johnson HO. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ Sci Technol 2001;35(4):766–70.

[47] Lau C, Strynar MJ, Lindstrom AB, Hanson RG, Thibodeaux JR, Barton HA. Pharmacokinetic evaluation of perfluorooctanoic acid in the mouse. The Toxicologist 2005;84(S-1):252.

[48] Grasty RC, Bjork JA, Wallace KB, Lau CS, Rogers JM. Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the perinatal rat. Birth Defects Res B Dev Reprod Toxicol 2005;74(5):405–16.

[49] Walther FJ, Waring AJ, Sherman MA, Zasadzinski JA, Gordon LM. Hydrophobic surfactant proteins and their analogues. Neonatology 2007;91(4):303–10.

[50] Zoetis T, Hurtt ME. Species comparison of lung development. Birth Defects Res B Dev Reprod Toxicol 2003;68(2):121–4.

[51] Lehmler HJ, Xie W, Bothun GD, Bummer PM, Knutson BL. Mixing of perfluorooctanesulfonic acid (PFOS) potassium salt with dipalmitoyl phosphatidylcholine (DPPC). Colloids Surf B Biointerfaces 2006;51(1):25–9.

[52] Xie W, Kania-Korwel I, Bummer PM, Lehmler HJ. Effect of potassium perfluorooctanesulfonate, perfluorooctanoate and octanesulfonate on the phase transition of dipalmitoylphosphatidylcholine (DPPC) bilayers. Biochim Biophys Acta 2007;1768(5):1299–308.

[53] Matyszewska D, Tappura K, Oradd G, Bilewicz R. Influence of perfluorinated compounds on the properties of model lipid membranes. J Phys Chem B 2007;111(33):9908–18.
[54] Gordon SC, Schurch S, Amrein M, Schoel M. Effects of perfluorinated acids on pulmonary surfactant properties in vitro. The Toxicologist 2007;96(1):91.
[55] Maloney EK, Waxman DJ. Trans-activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. Toxicol Appl Pharmacol 1999;161(2):209–18.
[56] Shipley JM, Hurst CH, Tanaka SS, DeRoos FL, Butenhoff JL, Seacat AM, et al. Trans-activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based chemicals. Toxicol Sci 2004;80(1):151–60.
[57] Takacs ML, Abbott BD. Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicol Sci 2007;95(1):108–17.